Journal of Neuroscience Research 50:505–513 (1997)

Mini-Review

Cell and Molecular Neurobiology of Presenilins: A Role for the Endoplasmic Reticulum in the Pathogenesis of Alzheimer's Disease?

Mark P. Mattson* and Qing Guo
Sanders-Brown Research Center on Aging and Department of Anatomy and Neurobiology, University of Kentucky, Lexington

Mutations in genes encoding presenilin-1 (PS-1) and presenilin-2 (PS-2) cause many cases of autosomal dominant inherited forms of early-onset Alzheimer's disease (AD). PSs are expressed in neurons throughout the nervous system, with differences in abundance among cell populations. PS-1 and PS-2 each have six to eight transmembrane domains and are localized mainly in the endoplasmic reticulum (ER). PSs may interact with cytoskeletal proteins and β-amyloid precursor protein (APP) in ways consistent with roles in membrane trafficking and APP processing. Expression of mutant PSs in cultured cells and transgenic mice results in increased production of an amyloidogenic-cytotoxic form of amyloid β-peptide (Aβ). Neural cells expressing mutant PSs exhibit increased sensitivity to apoptosis induced by trophic factor withdrawal and Aβ. The proapoptotic action of mutant PSs involves perturbed calcium release from ER stores and increased levels of oxidative stress. PS mutations may also suppress neurotransmitter synthesis in cholinergic neurons, suggesting a role in regulation of neuronal phenotype. Homology of PSs with the *C. elegans* gene *sel*-12 and phenotypic similarities of PS-1 and Notch knockout mice suggest a developmental role for PSs in somitogenesis. Collectively, the emerging data suggest intriguing roles of PSs in neuronal plasticity and cell death and highlight the importance of the ER as a regulatory site involved in the pathogenesis of neuronal degeneration in AD. J. Neurosci. Res. 50:505–513, 1997. © 1997 Wiley-Liss, Inc.

Key words: amyloid β-peptide; amyloid precursor protein; apoptosis; calcium homeostasis; inositol trisphosphate; muscarinic cholinergic; nerve growth factor; reactive oxygen species; vitamin E

PRESENILIN MOLECULAR GENETICS AND ALZHEIMER'S DISEASE

Mutations responsible for many cases of autosomal dominant (100% penetrance), early-onset Alzheimer's disease (AD) were localized to chromosomes 14 and 1 (St. George-Hyslop et al., 1992; Levy-Lahad et al., 1995a). Two years ago the genes harboring the mutations were identified (Levy-Lahad et al., 1995b; Rogaev et al., 1995; Sherrington et al., 1995), and they are now referred to as presenilin-1 (PS-1; chromosome 14) and presenilin-2 (PS-2; chromosome 1). At least 41 mutations in PS-1 and 2 mutations in PS-2 have been identified in familial AD kindreds (see Hardy, 1997 for review); because most of the PS mutations have only been identified in single families, additional PS mutations will almost surely be identified in the future. With one exception, all the PS-1 and PS-2 mutations are missense mutations that result in a single amino acid substitution. The age of onset of AD in families harboring PS-1 mutations is quite young, ranging from 29 to 56 years, whereas the age of onset in cases with PS-2 mutations is somewhat older (40+ years). All of the missense mutations affect amino acids that are conserved in PS-1 and PS-2, and the vast majority of the mutations occur in or immediately adjacent to transmembrane domains (Fig. 1). Particularly striking are clusters of mutations in two domains, one which encodes putative transmembrane domain 2 (15 mutations) and another immediately adjacent to the hydrophilic loop domain (11 mutations). Obviously, the latter regions of the PSs are critical for the pathogenic mechanism(s) of the PSs and are also likely to be domains that mediate important physiological functions of PSs.

CELLULAR EXPRESSION AND SUBCELLULAR LOCALIZATION OF PRESENILINS

Immunohistochemical analyses indicate that PSs are widely expressed in the nervous system, as well as in

Contract grant sponsor: NIH; Contract grant numbers: NS30583, AG10836, AG05144, and AG05119.

*Correspondence to: Mark P. Mattson, 211 Sanders-Brown Building, University of Kentucky, Lexington, KY 40536-0230. E-mail: MMattson@aging.coa.uky.edu

Received 26 May 1997; Revised 4 August 1997; Accepted 4 August 1997

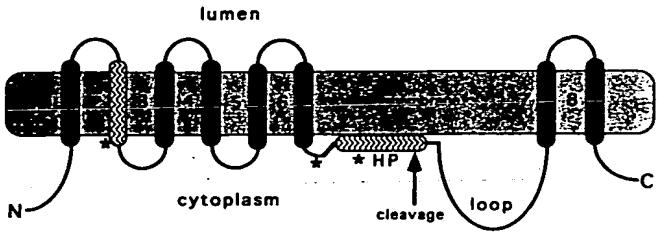

Fig. 1. Predicted structure of PS-1. PS-1 may have eight transmembrane domains with both the N- and C-termini, as well as a hydrophobic domain (HP) and a loop region, residing on the cytoplasmic side of the endoplasmic reticulum membrane. Full-length PS-1 can be enzymatically cleaved at the site adjacent to the loop region (arrow). Regions of major clusters of missense mutations (*) are located in transmembrane 2 and just N-terminal to the cleavage site. Modified from Hardy (1997).

many other organ systems (Sherrington et al., 1995; Levy-Lahad et al., 1995b). Within the brains of rodents, primates, and humans PSs are expressed at high levels in neurons, and there is considerable cellular colocalization of PS-1 and PS-2 (Cook et al., 1996; Elder et al., 1996; Kovacs et al., 1996; Lee et al., 1996; Suzuki et al., 1996; Lah et al., 1997). PS levels are particularly high in the pyramidal neurons of the hippocampus compared with dentate granule cells and neocortical cell populations (Page et al., 1996); PS-1 immunoreactivity is concentrated in cell bodies and dendrites, with lower levels present in axons (Elder et al., 1996; Lah et al., 1997). PSs are expressed during early stages of neurogenesis and differentiation in the rodent brain (Berezovska et al., 1997) and are also expressed in both neurons and astrocytes in primary dissociated cell cultures of embryonic mouse (Cribbs et al., 1996) and human (Fig. 2) brain. Excitotoxic lesions cause a loss of PS-1, consistent with expression primarily in neurons (Page et al., 1996). Cellular signaling pathways that may modulate expression of PSs remain to be identified. However, 5′ upstream regions of the PS-1 gene have been sequenced and reveal several potential transcription regulatory elements, including several STAT elements (Rogaev et al., 1997).

Immunocytochemical analyses of AD and control brains have shown that PS-1 is present in both nonvulnerable and vulnerable neurons, with levels being lower in neurofibrillary tangle-bearing neurons and neuritic plaques compared with undamaged neurons (Uchihara et al., 1996; Giannakopoulos et al., 1997; Weber et al., 1997). In cultured neural and nonneuronal cells PS-1 localizes to subcellular compartments and appears to be at particularly high levels in the endoplasmic reticulum (ER; Cook et al., 1996; Guo et al., 1996; Kovacs et al., 1996; Fig. 2). Walter et al. (1996) used confocal laser scanning microscopy and double-labeling using antibodies to PS-1 and the ER-specific protein grp78 to provide evidence that PS-1 and PS-2 are localized to the ER. In the latter study it was also shown that very little PS-1 or PS-2 is present in Golgi. Taken together with the predicted structure of PSs (see next section), the available data suggest that presenilins are concentrated in ER membranes in neurons.

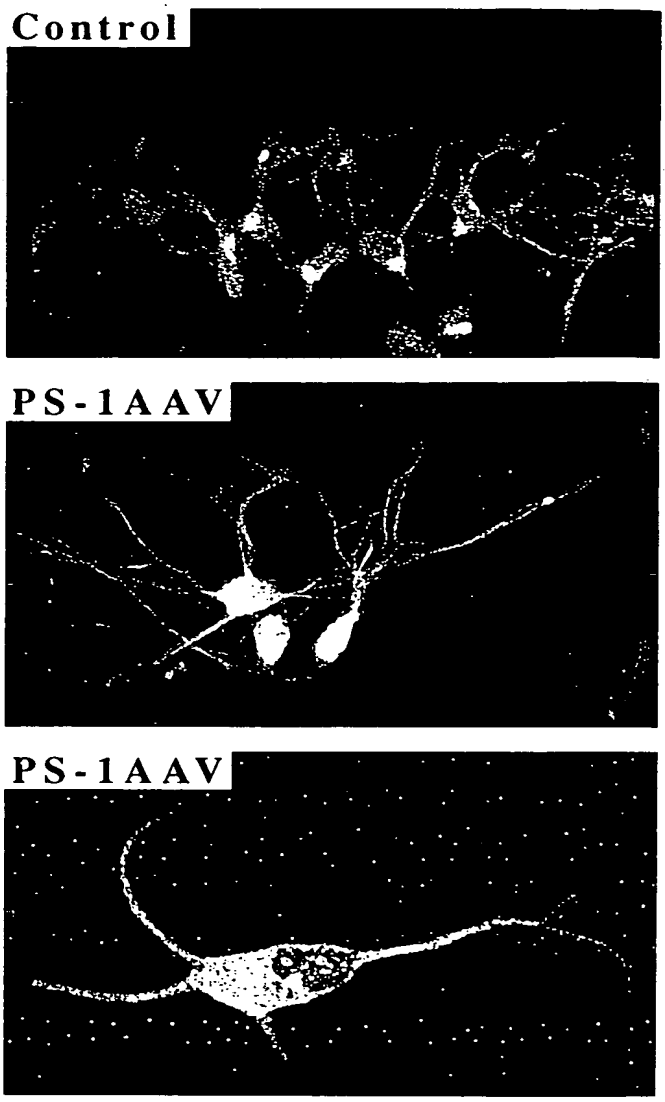

Fig. 2. Subcellular localization of PS-1 in cultured embryonic human cerebral cortical neurons. Confocal laser scanning microscope images of PS-1 immunoreactivity in control neurons (upper) and neurons transfected with wild-type PS-1 by using an adeno-associated virus (AAV) vector. Note localization of PS-1 in perinuclear organelles.

STRUCTURE AND MEMBRANE TOPOLOGY OF PRESENILINS

PS-1 and PS-2 have 67% identity at the amino acid level. Comparisons of the amino acids sequences of PSs with other known proteins and biochemical analyses of PS-1 and PS-2 suggest the presence of several functional domains. Some of the transmembrane domains in PSs have considerable homology to domains present in calcium channels (Fig. 1). On the basis of hydrophobicity analyses of the deduced amino acid sequences, Sherrington et al. (1995) and Levy-Lahad et al. (1995b) proposed that PS-1 and PS-2 contain seven transmembrane domains. This prediction placed the N- and C-termini of the proteins on opposite sides of the membrane. Doan et al. (1996) used two approaches to determine the topology of PS-1. First, they determined whether the putative transmembrane sequences were sufficient to move a protease-sensitive substrate across a lipid bilayer. Second, they used antibodies that recognize specific epitopes in PS-1 in immunostaining studies of permeabilized cultured CHO cells expressing chimeric proteins consisting of the C-terminus of β-amyloid precursor protein (APP) containing the Swedish double mutation and a putative transmembrane domain of PS-1. The results indicate that both the N- and C-termini are located on the cytosolic side of intracellular membranes; the loop domain is also located on the cytoplasmic side of the membrane (Fig. 1). PS-1 is predicted to have eight transmembrane domains. Similar predictions as to the membrane topology of presenilins came from the work of Li and Greenwald (1996) in their studies of the *C. elegans* presenilin homolog *sel*-12. The latter studies used *sel*-12:LacZ hybrid proteins, in which LacZ was placed after each of 10 hydrophobic regions predicted bases on hydrophobicity analyses. On the other hand, using a chimeric PS-1 in which a reporter protein containing three artificial glycosylation sites was fused to PS-1, Lehmann et al. (1997) provided evidence that PS-1 has a six transmembrane domain structure.

WHAT ARE THE NORMAL FUNCTIONS OF PRESENILINS?

At the present time the normal functions of PS-1 and PS-2 have not been established, and speculation as to their function(s) has come largely from knowledge of their structure, cellular expression, and subcellular localization. PS-1 and PS-2 have considerable homology to two *C. elegans* genes called *spe*-4 and *sel*-12 (Levitan and Greenwald, 1995; Levitan et al., 1996). *spe*-4 functions in spermatogenesis by regulating protein trafficking in the Golgi, whereas *sel*-12 plays a role in the process of egg laying by a mechanism involving the Notch signaling pathway. Patterns of expression of PS-1 and Notch in the developing rodent nervous system are very similar, being high during neurogenesis and decreasing as the embryo develops (Williams et al., 1995; Berezovska et al., 1997), suggesting the potential for functional interactions of PS-1 and Notch. Interestingly, human PS-1 can rescue defective egg laying resulting from mutations in *sel*-12, strongly suggesting similar functions of PSs and *sel*-12. Recently, PS-1 knockout mice were generated (Wong et al., 1997); they manifest defects in body segmentation similar to Notch mutant mice (Conlon et al., 1995) and die of cerebral hemorrhage late in gestation. Clearly, further studies of functions of Notch in neurons will provide valuable insight into the normal functions of PSs in the nervous system.

Several laboratories have shown that PS-1 is endoproteolytically processed in a manner suggesting a physiological role for such processing (Thinakarin et al., 1996; Mercken et al., 1996). In most cell types full-length (46–55 kDa) PSs are cleaved within exon 9 (loop domain), resulting in a 17–20 kDa C-terminal fragment and a 25–35 kDa N-terminal fragment. Tanzi and coworkers (1997) have shown that in the case of PS-2 the C-terminal fragment is found in the detergent-insoluble fractions, suggesting a cytoskeletal association. These findings raise the possibility that PS cytoplasmic domains may interact with the cytoskeleton, which could influence a variety of cellular processes including vesicle trafficking and ER calcium regulation, for example. In addition, Busciglio et al. (1997) described an association between the C-terminal fragment of PS-1 and neurofibrillary tangles, suggesting an interaction between PSs and the cytoskeletal elements (possibly tau protein) in AD. The mechanisms controlling proteolytic processing of PSs and the physiological role of such processing are unknown. Because many physiological processes are regulated by protein phosphorylation, several laboratories have been characterizing phosphorylation of PSs. Walter et al. (1996) reported that PS-2 is selectively phosphorylated on serine residues in an acidic N-terminal domain that is not present in PS-1. These findings suggest differences in the physiological regulation and/or function of PS-1 and PS-2; elucidating the identity of the kinase(s) and phosphatase(s) responsible for regulation of PS-2 phosphorylation will be of considerable interest.

Weidemann et al. (1997) reported that PS-1 interacts with APP in a noncovalent manner and provided evidence that this association could occur in ER; in addition, overexpression of PS-2 caused a decrease in APP secretion, suggesting a role for PS-2 in APP trafficking or proteolytic processing. Walter et al. (1996) also showed that PS-1 and PS-2 are localized in the ER, and to a lesser extent in the Golgi, further suggesting roles for PSs in APP processing (Fig. 3). Such actions of PSs

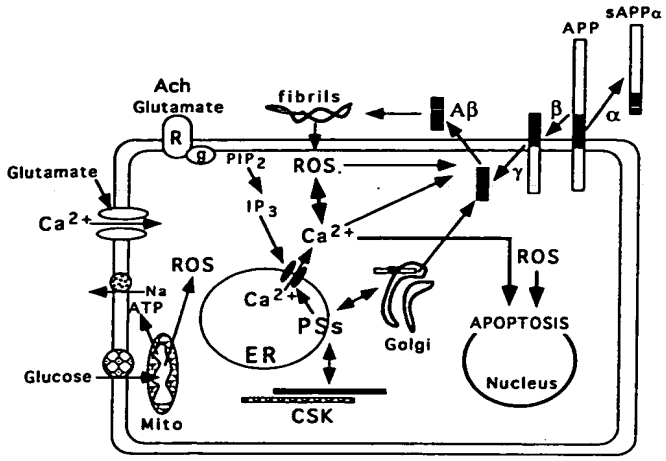

Fig. 3. Possible mechanisms underlying normal functions of PSs and the neurodegeneration-promoting actions of PS mutations in Alzheimer's disease. PS-1 and PS-2 (PSs) are located in the membrane of the endoplasmic reticulum (ER). PSs may play roles in the regulation of calcium release from ER stores, possibly by modulating the activity of inositol triphosphate (IP_3) receptors. By interacting with proteins in other cell membranes, such as the Golgi apparatus, or with cytoskeletal proteins (CSK) or β-amyloid precursor protein (APP), PSs may play roles in membrane trafficking and processing of proteins, including APP. Based on this model, there are several possible mechanisms whereby PS mutations promote neuronal degeneration in AD. By interacting with APP or altering APP trafficking, PS mutations may increase γ-secretase cleavage of APP, resulting in increased production of Aβ1-42, which then forms neurotoxic fibrils. By enhancing calcium release from ER, PS mutations sensitize neurons to apoptosis induced by cellular stress resulting from activation of glutamate receptors, metabolic compromise, and exposure to amyloid β-peptide (Aβ), for example. Mutant PSs have also been shown to enhance accumulation of reactive oxygens species (ROS), which could account for the increased oxidative stress in neurons evident in AD brain. Ach, acetylcholine; Mito, mitochondria.

are consistent with the known function of the PS homologue *sel*-4 in *C. elegans*.

A role for PSs in neuronal ER calcium signaling is suggested by recent calcium imaging studies, which showed that agonist-induced calcium release from ER is enhanced in PC12 cells expressing mutant PS-1 (Guo et al., 1996; Figs. 3 and 4A). The altered ER calcium regulation could, in principle, result from direct functional interactions of PS-1 with ER membrane proteins, such as the IP_3 receptor or Ca^{2+}-ATPase, or from effects on other systems that indirectly influence ER calcium homeostasis. Calcium release in response to thapsigargin, an inhibitor of the ER Ca^{2+}-ATPase was also enhanced in PC12 cells expressing mutant PS-1, suggesting that the primary mechanism of action of mutant PS-1 did not involve inhibition of the Ca^{2+}-ATPase (Guo et al., 1996). It is not known whether wild-type PSs serve a normal function in regulation of neuronal calcium homeostasis. However, given the key roles of calcium in regulating major developmental processes and in neurodegenerative disorders (see Mattson, 1992 for review), further studies of possible roles of PSs in calcium signaling are warranted. Finally, although PSs are present at high levels in the ER, and the major focus of current studies are on PS actions in this organelle, the possibility of major sites of PS function in other cellular compartments where they may be present at lower levels should not be overlooked.

MECHANISMS WHEREBY PRESENILIN MUTATIONS RESULT IN ALZHEIMER'S DISEASE

Deposits of Aβ (plaques) and neuronal degeneration, which manifests as synapse loss and neurofibrillary tangles, are defining features of AD (Selkoe, 1994). The question of how mutations in PSs lead to plaques, synapse loss, and neuronal death merits intense investigation because the answer will lead to a fuller understanding of AD pathogenesis and to novel preventative and therapeutic approaches. At present, there are data accumulating that support three different hypotheses: 1) PS mutations result in altered βAPP processing and increased levels of Aβ and/or the 1-42 form of Aβ; 2) PS mutations promote apoptotic cell death pathways; and 3) PS mutations cause aberrant subcellular (ER) calcium regulation which promotes excitotoxic and apoptotic cascades. These hypotheses are not mutually exclusive and may all prove to be, more or less, correct.

Altered β-APP Processing

Amyloid β-peptide (Aβ) is a 40–42 amino acid peptide that is generated by enzymatic cleavage of the β-amyloid precursor protein (APP), a transmembrane protein (Fig. 3). Although the enzymes responsible for cleavage at the N-terminus (β-secretase) and C-terminus (γ-secretase) of Aβ have not been identified, considerable information has accrued concerning mechanisms of APP processing (see Selkoe, 1994; Mattson, 1997a for review). Mutations in APP are linked to a small percentage of cases of autosomal, dominant inherited AD, and these mutations, when expressed in cultured cells or transgenic mice, result in increased production of the long form of Aβ (Aβ1-42), which has particular propencity to form insoluble amyloid fibrils (Younkin, 1995). Aβ1-42 appears to be the predominant form of Aβ deposited in the brain in AD, and it was therefore logical to test the hypothesis that PS mutations alter APP processing in a manner similar to APP mutations. Scheuner et al. (1996)

reported that, indeed, plasma from individuals bearing PS mutations had significantly elevated levels of Aβ1-42 and that fibroblasts from the carriers produce and release more Aβ1-42 than do fibroblasts from noncarriers. Moreover, cultured cells and transgenic mice overexpressing mutant PS-1 (but not wild-type PS-1) exhibit increased levels of Aβ1-42 (Duff et al., 1996; Borchelt et al., 1996). The mechanism whereby PS mutations increase Aβ1-42 levels is not known; PSs might participate directly in APP processing or they might influence other metabolic processes that secondarily affect APP processing. For example, we have proposed that a primary effect of PS mutations is to increase subcellular (oxidative and/or ionic) stress, which, in turn, leads to altered APP processing (Guo et al., 1997). In any case, the subcellular localization of PSs in the ER is providing important clues as to their roles in APP processing.

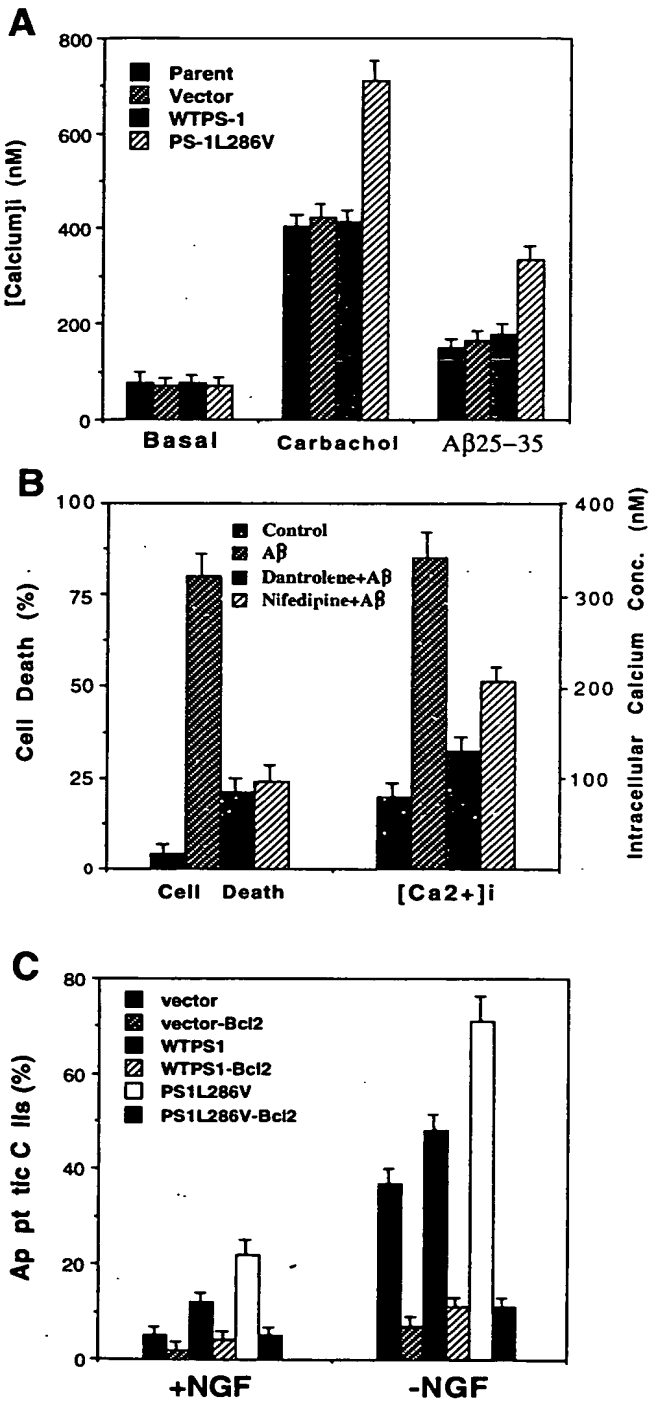

Fig. 4. Expression of mutant PS-1 in neural cells results in perturbed ER calcium regulation which is linked to increased vulnerability to apoptosis. **A:** Comparison of peak $[Ca^{2+}]_i$ responses to carbachol in different PC12 cell lines: untransfected parent cell line; vector transfected lines; lines overexpressing wild-type (WT) PS-1, and lines expressing mutant (L286V) PS-1. Values are means and SD of determinations made in four separate cultures (18–24 cells per culture). **B:** Cultures of PC12 cells expressing mutant (L286V) PS-1 were pretreated for 2 hr with vehicle (Control and Aβ), 1 μM dantrolene, or 1 μM nifedipine. Cultures were then exposed to 50 μM Aβ25-35 for either 4 hr (for measurement of $[Ca^{2+}]_i$; fura-2 imaging) or 24 hr (for quantification of cell survival; LDH release assay). Values are means and SD of determinations made in four cultures for $[Ca^{2+}]_i$ measurements (15–25 cells per culture) or 6–12 separate cultures for cell survival analysis. **C:** Following induction of PS-1 expression by removal of tetracycline, cultures of differentiated PC12 cells of the indicated lines were incubated for 48 hr in serum-free medium containing or lacking NGF, and the percentage of cells exhibiting apoptotic nuclei was quantified. Values are means and SEM of determinations made in at least four separate cultures. vector, vector-transfected control cell lines; WTPS1, wild-type PS-1; PS1L286V, PS-1 containing the L286V mutation. Modified from Guo et al., 1996, 1997.

Increased Sensitivity to Oxidative Stress-Induced Apoptosis

Apoptosis and necrosis are two morphologically distinct forms of cell death that appear to have both distinct and shared mechanistic underpinnings. Cells undergoing apoptosis shrink and exhibit nuclear chromatin condensation and DNA fragmentation, whereas necrotic cells swell and lyse. Apoptosis has been referred to as "programmed cell death" because macromolecular synthesis inhibitors can suppress the death in many instances, suggesting the requirement for production of "killer" proteins (Thompson, 1995). However, it is also clear that apoptosis can result from insufficient levels of activation of antiapoptotic signaling pathways (Mattson et al., 1996). In general, cells that die as the result of subtle and protracted exposure to adverse conditions

undergo apoptosis, whereas cells subjected to sudden and severe insults will die by necrosis. Data from studies of postmortem AD brain tissue (Su et al., 1994; Smale et al., 1995) and culture paradigms of neuronal death relevant to AD, such as exposure to Aβ (Loo et al., 1993; Kruman et al., 1997), suggest that neuronal apoptosis occurs in AD. When taken together with the observation that PS-2 is homologous to ALG-3, a mouse protein that protects T-hybridoma cells against apoptosis induced by *fas* ligand (Vito et al., 1996), possible actions of PSs in apoptotic or antiapoptotic pathways were explored.

It was reported that overexpression of wild-type PS-2 in PC12 cells enhances apoptosis induced by Aβ and trophic factor withdrawal (Wolozin et al., 1996) or exposure to staurosporine or hydrogen peroxide (Deng et al., 1996). The proapoptotic action of wild-type PS-2 in neural cells somewhat contradicts the data from studies of ALG-3 in hybridoma cells. However, it was also reported that mutant PS-2 has enhanced apoptotic activity compared with wild-type PS-2 (Wolozin et al., 1996), suggesting a possible mechanism of action in AD. The latter findings corroborate a previous study that showed that expression of mutant PS-1 in cultured PC12 cells sensitizes them to apoptosis induced by Aβ (Guo et al., 1996). In the case of PS-1, however, overexpression of wild-type PS-1 has little or no proapoptotic effects (Guo et al., 1996, 1997). In the case of PS-2, the mechanism underlying a proapoptotic effect is unknown. In the case of mutant PS-1 the apoptotic mechanism may involve perturbation of ER calcium signaling and calcium overload because the proapoptotic action is suppressed by dantrolene and nifedipine, compounds that block calcium release from ER and calcium influx through plasma membrane voltage-dependent channels, respectively (Guo et al., 1996, 1997; Fig. 4B).

By controlling the expression of wild-type and mutant PS-1 in PC12 cells by using a tetracycline-responsive promoter, we were able to differentiate the cells into a neuron-like phenotype by exposure to nerve growth factor (NGF) in the absence of PS-1 expression and then turn on PS-1 expression in the differentiated cells (Guo et al., 1997). Expression of mutant (L286V) in differentiated PC12 cells greatly increased their vulnerability to NGF withdrawal-induced apoptosis (Fig. 4C). PC12 cells coexpressing mutant PS-1 and the antiapoptotic gene product Bcl-2 were resistant to apoptosis induced by NGF withdrawal or exposure to Aβ. NGF withdrawal induced an increase in the level of cellular peroxides in PC12 cells, and this marker of oxidative stress was enhanced in cells expressing mutant PS-1 (Guo et al., 1997). Bcl-2 suppressed the adverse effect of mutant PS-1 on levels of cellular oxidative stress. Collectively, these data suggest that PS mutations may promote neuronal death by enhancing levels of oxidative stress.

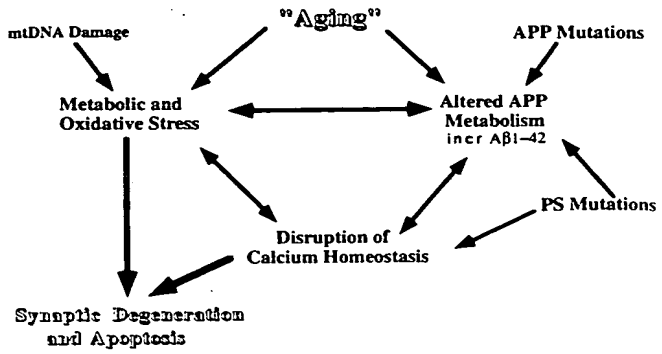

Fig. 5. Flow chart of neurodegenerative pathways in Alzheimer's disease. A major risk factor for AD is "aging," which is associated with increases in cellular oxidative stress, mitochondrial DNA (mtDNA) damage, and metabolic impairment. Oxidative stress can impair neuronal calcium homeostasis, thereby promoting excitotoxic synaptic degeneration and apoptotic cell death. APP mutations result in altered enzymatic processing of APP leading to increased production of amyloid β-peptide (particularly Aβ1-42), which induces oxidative stress and disrupts calcium homeostasis in neurons. Conversely, oxidative stress and elevated intracellular calcium levels can alter APP processing in favor of increased Aβ1-42 production, thus perpetuating a cyclic degenerative cascade. Presenilin (PS) mutations may promote neuronal degeneration by altering APP metabolism and/or by disrupting endoplasmic reticulum calcium homeostasis and inducing oxidative stress.

Considerable data suggest that increased cellular oxidative stress contributes greatly to the neurodegenerative process in AD. The evidence includes the presence of oxidatively damaged proteins, lipids, and DNA in association with neurofibrillary tangles and senile plaques, and the ability of Aβ to induce oxidative stress in neurons (see Benzi and Moretti, 1995; Mattson et al., 1996 for review). The mechanism of Aβ-induced apoptosis appears to involve increased membrane lipid peroxidation, which results in impairment of ion-motive ATPases and glucose transporters (Mark et al., 1995, 1997a,b; Goodman et al., 1996; Kruman et al., 1997). Expression of mutant PS-1 in PC12 cells results in increased oxidative stress as indicated by peroxide accumulation and mitochondrial dysfunction following trophic factor withdrawal or exposure to Aβ (Guo et al., 1997). Indeed, the latter study showed that antioxidants that suppress membrane lipid peroxidation, such as vitamin E and propyl gallate, can protect neural cells against the proapoptotic actions of PS-1 mutations. The increased oxidative stress may be secondary to perturbed ER calcium regulation or may result from yet-to-be identified, direct effects of PS-1 mutations on free radical metabolism. The adverse effects of PS mutations on neuronal sensitivity to oxidative stress-induced apoptosis likely interact synergistically or additively with mitochondrial DNA damage and impaired

energy metabolism, which appear to be prevalent in many if not all AD cases (Fig. 5; Mattson, 1997b).

Altered Calcium Homeostasis

Data obtained from studies of AD brain tissue and cell culture and animal studies of AD-relevant neurodegenerative paradigms suggest important roles for perturbed neuronal calcium homeostasis in the pathogenesis of neuronal dysfunction and death in AD (see Mattson et al., 1996; Mattson, 1997a for review). Examples include the following: neurofibrillary tangles and neuritic plaques exhibit evidence of increased calcium-activated protease activities (Nixon et al., 1994); Aβ disrupts neuronal calcium homeostasis and increases neuronal vulnerability to excitotoxicity (Mattson et al., 1992; Mark et al., 1995); and insults that increase intraneuronal calcium levels induce cytoskeletal alterations in hippocampal neurons (in cell culture and in vivo) similar to those seen in neurofibrillary tangles (Mattson, 1990; Stein-Behrens et al., 1994). Mutations in β-APP that are causally linked to a small percentage of cases of inherited AD may promote uncontrollable increases in intracellular free calcium levels $[Ca^{2+}]_i$ by at least two mechanisms (Figs. 3 and 5; Mattson, 1997a). First, by increasing the levels of total Aβ, and/or of the 1-42 form of Aβ, the mutations would lead to increased Aβ-mediated disruption of calcium homeostasis. Second, the mutations may decrease levels of secreted forms of APP (sAPP-α) which have been shown to stabilize neuronal calcium homeostasis and protect neurons against excitotoxic, metabolic, and oxidative insults, including Aβ toxicity (Mattson et al., 1993; Furukawa et al., 1996).

Several lines of evidence suggest that PSs may modulate neuronal calcium homeostasis and, importantly, that PS mutations may perturb subcellular calcium homeostasis in ways that promote neuronal degeneration. Studies of cultured fibroblasts from familial AD patients linked to chromosome 14 (now known to harbor PS-1 mutations) exhibited increased calcium release from intracellular stores in response to bradykinin and bombesin, agonists with receptors linked to the inositol triphosphate (IP_3) signaling pathway (Ito et al., 1994). PC12 neural cells expressing mutant PS-1 (L286V) exhibit increased calcium release from ER when stimulated with the muscarinic receptor agonist carbachol or with bradykinin (Guo et al., 1996). Elevations of $[Ca^{2+}]_i$ and cell death induced by Aβ were also greatly increased in PC12 cells harboring PS-1 mutations (Guo et al., 1996). The latter studies showed that dantrolene, a blocker of calcium release from ER, protected PC12 cells against the adverse effects of PS-1, suggesting that the perturbed calcium homeostasis conferred by mutant PS-1 played a key role in increased vulnerability to Aβ. Perturbed calcium homeostasis, together with enhanced oxidative stress, appear to contribute greatly to the proapoptotic actions of PS mutations (Guo et al., 1997). The possibility that perturbed ER calcium homeostasis is sufficient to account for both the altered β-APP processing and proapoptotic actions of mutant PSs merits consideration. Levels of the type-3 IP_3 receptor are increased in lymphocytes induced to undergo apoptosis, and antisense oligonucleotides to the IP_3 receptor prevent apoptosis (Khan et al., 1996). Levels of acylphosphatase, an enzyme that modulates the activity of Ca^{2+}-ATPase were reported to be increased in fibroblasts from patients bearing PS-1 mutations (Liguri et al., 1996). Haug et al. (1996) reported that levels of IP_3 receptors are decreased in cerebral cortical tissue from AD patients. Considerable work will be required to establish the interrelationships of PSs, ER signaling, and apoptosis.

OTHER MECHANISMS

Although the mechanisms whereby PS mutations lead to neuronal death are a central issue, it is also important to consider the impact of PS mutations on neuronal function in the absence of overt neuronal degeneration. Levels of choline acetyltransferase (ChAT), a key enzyme in the biosynthetic pathway for the neurotransmitter acetylcholine, are markedly reduced in PC12 cells expressing mutant PS-1 compared with control PC12 cell lines and to lines overexpressing wild-type PS-1 (Pedersen et al., 1997). The adverse effect of mutant PS-1 on cholinergic phenotype occurred both in undifferentiated cells and cells differentiated into a neuron-like phenotype. The mechanism whereby PS-1 mutations result in reduced ChAT activity are not known but may be related to the well-documented deficits in ChAT and acetylcholine in basal forebrain cholinergic neurons and their cortical and hippocampal targets in AD.

ACKNOWLEDGMENTS

We thank K. Furukawa and W. Pedersen for contributions to original research described herein. This work was supported by grants to M.P.M. from the NIH (NS30583, AG10836, AG05144 and AG05119) and the Alzheimer's Association.

REFERENCES

Benzi G, Moretti A (1995): Are reactive oxygen species involved in Alzheimer's disease? Neurobiol Aging 16:661–674.

Berezovska O, Xia MQ, Page K, Wasco W, Tanzi RE, Hyman BT (1997): Developmental regulation of presenilin mRNA expression parallels notch expression. J Neuropathol Exp Neurol 56:40–44.

Borchelt DR, Thinakaran G, Eckman CB, Lee MK, Davenport F, Ratovitsky T, Prada C-M, Kim G, Seekins S, Yager D, Slunt HH, Wang R, Seeger M, Levey AI, Gandy SE, Copeland NG, Jenkins NA, Price DL, Younkin SG, Sisodia SS (1996): Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1-42/1-40 ratio in vitro and in vivo. Neuron 17:1005–1013.

Busciglio J, Hartmann H, Lorenzo A, Wong C, Baumann K, Sommer B, Staufenbiel M, Yankner BA (1997): Neuronal localization of presenilin-1 and association with amyloid plaques and neurofibrillary tangles in Alzheimer's disease. J Neurosci 17:5101–5107.

Conlon RA, Reaume AG, Rossant J (1995): Notch1 is required for the coordinate segmentation of somites. Development 121:1533–1545.

Cook DB, Sung JC, Golde TE, Felsenstein KM, Wojczyk BS, Tanzi RE, Trojanowski JQ, Lee VMY, Doms RW (1996): Expression and analysis of presenilin 1 in a human neuronal system: Localization in cell bodies and dendrites. Proc Natl Acad Sci USA 93:9223–9228.

Cribbs DH, Chen L, Bendle SM, La Ferla FM (1996): Widespread neuronal expression of the presenilin-1 early-onset Alzheimer's disease in the murine brain. Am J Pathol 148:1797–1806.

Deng G, Pike CJ, Cotman CW (1996): Alzheimer-associated presenilin-2 confers increased sensitivity to apoptosis in PC12 cells. FEBS Lett 397:50–54.

Doan A, Thinakaran G, Borchelt DR, Slunt HH, Ratovistsky T, Podlisny M, Selkoe DJ, Seeger M, Gandy SE, Price DL, Sisodia SS (1996): Protein topology of presenilin 1. Neuron 17:1023–1030.

Duff K, Eckman C, Zehr C, et al. (1996): Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. Nature 383:710–713.

Elder GA, Tezapsidis N, Carter J, Shioi J, Bouras C, Li D, Johnston JM, Efthimiopoulos S, Friedrich VL, Robakis NK (1996): Identification and neuron specific expression of the S182/presenilin 1 protein in human and rodent brains. J Neurosci Res 45:308–320.

Furukawa K, Sopher B, Rydel RE, Begley JG, Martin GM, Mattson MP (1996): Increased activity-regulating and neuroprotective efficacy of α-secretase-derived secreted APP is conferred by a C-terminal heparin-binding domain. J Neurochem 67:1882–1896.

Giannakopoulos P, Bouras C, Kovari E, Shioi J, Tezapsidis N, Hof PR, Robakis NK (1997): Presenilin-1 immunoreactive neurons are preserved in late-onset Alzheimer's disease. Am J Pathol 150:429–436.

Goodman Y, Bruce AJ, Cheng B, Mattson MP (1996): Estrogens attenuate and corticosterone exacerbates excitotoxicity, oxidative injury and amyloid β-peptide toxicity in hippocampal neurons. J Neurochem 66:1836–1844.

Guo Q, Furukawa K, Sopher BL, Pham DG, Xie J, Robinson N, Martin GM, Mattson MP (1996): Alzheimer's PS-1 mutation perturbs calcium homeostasis and sensitizes PC12 cells to death induced by amyloid β-peptide. Neuroreport 8:379–383.

Guo G, Sopher BL, Pham DG, Furukawa K, Robinson N, Martin GM, Mattson MP (1997): Alzheimer's presenilin mutation sensitizes neural cells to apoptosis induced by trophic factor withdrawal and amyloid β-peptide: Involvement of calcium and oxyradicals. J Neurosci 17:4212–4222.

Hardy J (1997): Amyloid, the presenilins and Alzheimer's disease. Trends Neurosci 20:154–159.

Haug LS, Ostvold AC, Cowburn RF, Garlind A, Winblad B, Bogdanovich N, Walaas SI (1996): Decreased inositol (1,4,5)-trisphosphate receptor levels in Alzheimer's disease cerebral cortex: Selectivity of changes and possible correlation to pathological severity. Neurodegeneration 5:169–176.

Ito E, Oka K, Etcheberrigaray R, Nelson TJ, McPhie DL, Tofel-Grehl B, Gibson GE, Alkon DL (1994): Internal Ca^{2+} mobilization is altered in fibroblasts from patients with Alzheimer disease. Proc Natl Acad Sci USA 91:534–538.

Khan AA, Soloski MJ, Sharp AH, Schilling G, Sabatini DM, Li SH, Ross CA, Snyder SH (1996): Lymphocyte apoptosis: Mediation by increased type 3 inositol 1,4,5-trisphosphate receptor. Science 273:503–507.

Kovacs DM, Fausett HJ, Page KJ, Kim T-W, Moir RD, Merriam DE, Hollister RD, Hallmark OG, Mancini R, Felsenstein KM, Hyman BT, Tanzi RE, Wasco W (1996): Alzheimer-associated presenilins 1 and 2: Neuronal expression in brain and localization to intracellular membranes in mammalian cells. Nature Med 2:224–229.

Kruman I, Bruce-Keller AJ, Bredesen DE, Waeg G, Mattson MP (1997): Evidence that 4-hydroxynonenal mediates oxidative stress-induced neuronal apoptosis. J Neurosci 17:5097–5108.

Lah JJ, Heilman CJ, Nash NR, Rees HD, Yi H, Counts SE, Levey AI (1997): Light and electron microscopic localization of presenilin-1 in primate brain. J Neurosci 17:1971–1980.

Lee MK, Slunt HH, Martin LJ, Thinakaran G, Kim G, Gandy SE, Seeger M, Koo E, Price DL, Sisodia SS (1996): Expression of presenilin 1 and 2 (PS1 and PS2) in human and murine tissues. J Neurosci 16:7513–7525.

Lehmann S, Chiesa R, Harris DA (1997): Evidence for a six-transmembrane domain structure of presenilin 1. J Biol Chem 272:12047–12051.

Levitan D, Greenwald I (1995): Facilitation of lin-12-mediated signalling by sel-12, a *Caenorhabditis elegans* S182 Alzheimer's disease gene. Nature 377:351–354.

Levitan D, Doyle TG, Brousseau D, Lee MK, Thinakarin G, Slunt HH, Sisodia SS, Greenwald I (1996): Assessment of normal and mutant human presenilin function in Caenorhabditis elegans. Proc Natl Acad Sci USA 93:14940–14944.

Levy-Lahad E, Wijsman EM, Nemens E, Anderson L, Goddard KAB, Weber JL, Bird TD, Schellenberg GD (1995a): A familial Alzheimer's disease locus on chromosome 1. Science 269:970–973.

Levy-Lahad E, Wasco W, Poorkaj P, Romano DM, Oshima J, Pettingell WH, Yu C-E, Jondro PD, Schmidt SD, Wang K, Crowley AC, Fu Y-H, Guenette SY, Galas D, Nemens E, Wijsman EM, Bird TD, Schellenberg GD, Tanzi RE (1995b): Candidate gene for the chromosome 1 familial Alzheimer's disease locus. Science 269:973–977.

L'Hernault SW, Arduengo PM (1992): Mutation of a putative sperm membrane protein in *Caenorhabditis elegans* prevents sperm differentiation but not its associated meiotic divisions. J Cell Biol 119:55–68.

Li X, Greenwald I (1996): Membrane topology of the C. elegans SEL-12 presenilin. Neuron 17:1015–1021.

Liguri G, Cecchi C, Latorraca S, Pieri A, Sorbi S, Degl'Innocenti D, Ramponi G (1996): Alteration of acylphosphatase levels in familial Alzheimer's disease fibroblasts with presenilin gene mutations. Neurosci Lett 210:153–156.

Loo D, Copani A, Pike C, Whittemore E, Walencewicz A, Cotman CW (1993): Apoptosis is induced by β-amyloid in cultured central nervous system neurons. Proc Natl Acad Sci USA 90:7951–7955.

Mark RJ, Hensley K, Butterfield DA, Mattson MP (1995): Amyloid β-peptide impairs ion-motive ATPase activities: Evidence for a role in loss of neuronal Ca^{2+} homeostasis and cell death. J Neurosci 15:6239–6249.

Mark RJ, Lovell MA, Markesbery WR, Uchida K, Mattson MP (1997a): A role for 4-hydroxynonenal in disruption of ion homeostasis and neuronal death induced by amyloid β-peptide. J Neurochem 68:255–264.

Mark RJ, Pang Z, Geddes JW, Mattson MP (1997b): Amyloid β-peptide impairs glucose uptake in hippocampal and cortical neurons: Involvement of membrane lipid peroxidation. J Neurosci 17:1046–1054.

Mattson MP (1990): Antigenic changes similar to those seen in neurofibrillary tangles are elicited by glutamate and calcium influx in cultured hippocampal neurons. Neuron 4:105–117.

Mattson MP (1992): Calcium as sculptor and destroyer of neural circuitry. Exp Gerontol 27:29–49.

Mattson MP (1997a): Cellular actions of β-amyloid precursor protein, and its soluble and fibrillogenic peptide derivatives. Physiol Rev 77:1081–1132.

Mattson MP (1997b): Mother's legacy: Mitochondrial DNA mutations and Alzheimer's disease. Trends Neurosci 20:373–375.

Mattson MP, Cheng B, Davis D, Bryant K, Lieberburg I, Rydel RE (1992): β-amyloid peptides destabilize calcium homeostasis and render human cortical neurons vulnerable to excitotoxicity. J Neurosci 12:376–389.

Mattson MP, Cheng B, Culwell A, Esch F, Lieberburg I, Rydel RE (1993): Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of β-amyloid precursor protein. Neuron 10:243–254.

Mattson MP, Furukawa K, Bruce AJ, Mark RJ, Blanc EM (1996): Calcium homeostasis and free radical metabolism as convergence points in the pathophysiology of dementia. In Wasco W, Tanzi RE (eds) "Molecular Mechanisms of Dementia." Humana Press, pp. 103–143.

Mercken M, Takahashi H, Honda T, Sato K, Murayama M, Nakazato Y, Noguchi K, Imahori K, Takashima A (1996): Characterization of human presenilin 1 using N-terminal specific monoclonal antibodies: Evidence that Alzheimer mutations affect proteolytic processing. FEBS Lett 389:297–303.

Nixon RA, Saito KI, Grynspan F, Griffin WR, Katayama S, Honda T, Mohan PS, Shea TB, Beermann M (1994): Calcium-activated neutral proteinase (calpain) system in aging and Alzheimer's disease. Ann NY Acad Sci 747:77–91.

Page K, Hollister R, Tanzi RE, Hyman BT (1996): In situ hybridization of presenilin 1 mRNA in Alzheimer's disease and lesioned rat brain. Proc Natl Acad Sci USA 93:14020–14024.

Pedersen W, Guo Q, Hartman BK, Mattson MP (1997): NGF-independent reduction of choline acetyltransferase activity in PC12 cells expressing mutant presenilin-1. J Biol Chem 272: 22397–22400.

Rogaev EI, Sherrington R, Rogaeva EA, Levesque G, Ikeda M, Liang Y, Chi H, Lin C, Holman K, Tsuda T, Mar L, Sorbi S, Nacmias B, Piacentini S, Amaducci L, Chumakov I, Cohen D, Lannfelt L, Fraser PE, Rommens JM, St. George-Hyslop PH (1995): Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. Nature 376:775–778.

Rogaev EI, Sherrington R, Wu C, Levesque G, Liang Y, Rogaeva EA, Ikeda M, Holman K, Lin C, Lukiw WJ, de Jong PJ, Fraser PE, Rommens JM, St George-Hyslop P (1997): Analysis of the 5′ sequence, genomic structure, and alternative splicing of the preenilin-1 gene associated with early onset Alzheimer's disease. Genomics 40:415–424.

St. George-Hyslop PH, Haines J, Rogaev E, Mortilla M, Vaula G, Pericak-Vance M, Foncin JF, Montesi M, Bruni A, Sorbi S, Rainero I, Pinessi L, Pollen D, Polnsky R, Nee L, Kennedy J, Macciardi F, Rogaeva E, Liang Y, Alexandrova N, Lukiw W, Schlumpf K, Tanzi R, Tsuda T, Farrer L, Cantu J-M, Duara R, Amaducci L, Bergamini L, Gusella J, Roses A, Crapper McLachlan D (1992): Genetic evidence for a novel familial Alzheimer's disease locus on chromosome 14. Nature Gen 2:330–334.

Scheuner D, Eckman C, Jensen M, Song X, Citron M, Suzuki N, Bird TD, Hardy J, Hutton M, Kukull W, Larson E, Levy-Lahad E, Viitanen M, Peskind E, Poorkaj P, Schellenberg G, Tanzi R, Wasco W, Lannfelt L, Selkoe D, Younkin S (1996): The amyloiod β protein deposited in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease. Nature Med 2:864–870.

Selkoe DJ (1994): Cell biology of the amyloid beta-protein precursor and the mechanism of Alzheimer's disease. Annu Rev Cell Biol 10:373–403.

Sherrington R, Rogaev EI, Liang Y, Rogaeva EA, Levesque G, Ikeda M, Chi H, Lin C, Li G, Holman K, Tsuda T, Mar L, Foncin J-F, Bruni AC, Montesi MP, Sorbi S, Rainero I, Pinessi L, Nee L, Chumakov I, Pollen D, Brookes A, Sansequ P, Polinsky RJ, Wasco W, Da Silva HAR, Haines JL, Pericak-Vance MA, Tanzi RE, Roses AD, Fraser PE, Rommens JM, St. George-Hyslop PH (1995): Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. Nature 375:754–760.

Smale G, Nichols NR, Brady DR (1995): Evidence for apoptotic cell death in Alzheimer's disease. Exp Neurol 133:225–230.

Stein-Behrens B, Mattson MP, Chang I, Yeh M, Sapolsky RM (1994): Stress excacerbates neuron loss and cytoskeletal pathology in the hippocampus. J Neurosci 14:5373–5380.

Su JH, Anderson AJ, Cummings B, Cotman CW (1994): Immunocytochemical evidence for apoptosis in Alzheimer's disease. Neuroreport 5:2529–2533.

Suzuki T, Nishiyama K, Murayama S, Yamamoto A, Sato S, Kanazawa I, Sakaki Y (1996): Regional and cellular presenilin 1 gene expression in human and rat tissues. Biochem Biophys Res Commun 219:708–713.

Tanzi R, Kovacs DM, Kim TW, Moir RD, Guenette SY, Wasco W (1997): The presenilin genes and their role in early-onset familial Alzheimer's disease. Alzheimer's Dis Rev 1:90–98.

Thinakarin G, Borchelt DR, Lee MK, Slunt HH, Spitzer L, Kim G, Ratovitsky T, Davenport F, Norstedt C, Seeger M, Hardy J, Levey A, Gandy SE, Jenkins NA, Copeland NG, Price DL, Sisodia SA (1996): Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. Neuron 17:181–190.

Thompson CB (1995): Apoptosis in the pathogenesis and treatment of disease. Science 267:1456–1462.

Uchihara T, el Hachimi HK, Duyckaerts C, Foncin JF, Fraser PE, Levesque L, St. George-Hyslop PH, Hauw JJ (1996): Widespread immunoreactivity of presenilin in neurons of normal and Alzheimer's disease brains: double-labeling immunohistochemical study. Acta Neuropathol 92:325–330.

Vito P, Lacan E, D'Adamio L (1996): Interfering with apoptosis: Ca^{2+}-binding protein ALG-2 and Alzheimer's disease gene ALG-3. Science 271:521–525.

Walter J, Capell A, Grunberg J, Pesold B, Schindzielorz A, Prior R, Podlinsy MB, Fraser P, St. George-Hyslop P, Selkoe DJ, Haass C (1996): The Alzheimer's disease-associated presenilins are differentially phosphorylated proteins located predominantly within the endoplasmic reticulum. Mol Med 2:673–691.

Weber LL, Leissring MA, Yang AJ, Glabe CG, Cribbs DH, LaFerla FM (1997): Presenilin-1 immunoreactivity is localized intracellularly in Alzheimer's disease brain, but not detected in amyloid plaques. Exp Neurol 143:37–44.

Weidemann A, Paliga K, Durrwang U, Czech C, Evin G, Masters CL, Beyreuther K (1997): Formation of stable complexes between two Alzheimer's disease gene products: Presenilin-2 and β-amyloid precursor protein. Nat Med 3:328–332.

Williams R, Lendahl U, Lardelli M (1995): Complementary and combinatorial pattern of Notch gene family expression during early mouse development. Mech Dev 53:357–368.

Wolozin B, Iwasaki K, Vito P, Ganjei JK, Lacana E, Sunderland T, Zhao B, Kusiak JW, Wasco W, D'Adamio L (1996): Participation of presenilin 2 in apoptosis: Enhanced basal activity conferred by an Alzheimer mutation. Science 274:1710–1713.

Wong PC, Zheng H, Chen H, Becher MW, Sirinathsinghji DJS, Trumbauer ME, Chen HY, Price DL, Van der Ploeg LHT, Sisodia SS (1997): Presenilin 1 is required for *Notch1* and *Dll1* expression in the paraxial mesoderm. Nature 387:288–292.

Younkin S (1995): Evidence that Aβ 42 is the real culprit in Alzheimer's disease. Ann Neurol 37:287–288.